G5

Cancld. 112. (new) The portion of a *Clostridium botulinum* toxin of Claim 110 encoded on a vector wherein the vector is included in a host cell.

Please cancel the following Claims:

45, 51 and 80.

REMARKS

The amendments to the claims have been made to more clearly claim and present the invention. The amendments to the claims are not made for a reason related to the statutory requirements of patentablility and/or the claims as a whole have not been narrowed. Hence, the amended claims are not subject to the rule set forth in *Festo Corp. v. Shoketsu Kinzoku Kogyo Kabushiki Co.*, 56 USPQ2d 1865 (Fed. Cir. 2000).

Rejection of Claims 42, 54-55, 79-80, 93-94 and 107-109 Under 35 U.S.C. § 112 second paragraph

The Examiner rejected Claims 42, 54-55, 79-80, 93-94 and 107-109 under 35 U.S.C. § 112 second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicants regards as the invention. In particular the Examiner states that "comprising" used in the claimed invention is a relative word that fails to define the structure of the claimed fusion protein molecule. The Examiner also indicates that the term "portion" is not definite and therefore renders the claims indefinite.

The term "comprising" is inclusive or open-ended and does not exclude additional, unrecited elements or method steps (MPEP §

2111.03). "Comprising" is commonly used in claims when referring to components that comprise a fusion protein. For example, see U.S. Patent No. 6,329,163, Claim 10; U.S. Patent No. 6,329,157, claims 1-17; U.S. Patent No. 6,326,150, Claims 1, 11, 16, 18 and 20 and claims 1 and 10 of U.S. Patent No. 5,919,665 which is related to the present patent application. The present use of the term "comprising" is consistent with accepted usage of the term and is therefore appropriate as used in the present claims. Additionally, applicants present herein new claims 110 to 112 directed to "A soluble, recombinant protein consisting essentially of a portion of a *Clostridium botulinum* wherein the portion ranges in size from four amino acid residues to the entire protein minus one amino acid of SEQ ID NO: 28" and these claims clearly overcome the rejection.

The specification leaves no doubt as to the meaning of the term "portion" where on page 17, lines 25-27 it is stated: "As used herein the term "portion" when in reference to a protein (as in "a portion of a given protein") refers to fragments of that protein. The fragments may range in size from four amino acid residues to the entire amino acid sequence minus one amino acid."

The meaning of the term "portion" has been included in the claims to obviate the Examiner's rejection.

Claim 97 is rejected as indefinite for reciting "comprising at least a portion of." The Examiner states that it is unclear how much of the Clostridium peptide is required. Applicants traverse the rejection. However, Claim 97 has been amended to delete "at least" to obviate the Examiner's rejection.

Claim 42 does not include either "comprising" or "portion" and accordingly, applicants submit that the present objection should be withdrawn.

Rejection of Claims 42, 54-55, 79-80, 93-94 and 107-109 Under 35 U.S.C. § 101

The Examiner rejected Claims 42, 54-55, 79-80, 93-94 and 107-109 under 35 U.S.C. § 101 stating that these Claims claim the same invention as that of Claims 1-10 of U.S. Patent No. 5,919,665. This is a statutory double patenting rejection.

35 U.S.C. 101 prevents patents from issuing on the same invention. As the court ruled in Miller v. Eagle Mfg. Co., 151 U.S. 186; In re Vogel, 164 USPQ 619; and In re Ockert, 114 USPQ 330: the same invention means identical subject matter.

During the telephonic interview of December 18, 2001, the Examiner suggested amending the claimed polypeptides to a specific amino acid sequence to overcome the statutory double patenting issue. Applicants respectfully submit that none of the pending Claims recite subject matter identical to that claimed in issued Patent 5,919,665. In particular, Claims 42, 54, 55 and 79 are broader than the issued Claims. Claims 93 and 94 specify "endotoxin free" and Claims 107, 108 and 109 specify covalent bonding and are therefore different than the issued Claims. In addition, applicants present herein new claims 110 to 112 in which a SEQ ID NO (SEQ ID NO: 28) is specified. These new Claims clearly overcome the rejection.

Rejection of Claims 42-43, 54-57, 79-80, 83, 86, 89-91, 93-94, 100, 103-105 and 107-109 Under 35 U.S.C. § 103(a)

The Examiner rejected Claims 42-43, 54-57, 79-80, 83, 86, 89-91, 93-94, 100, 103-105 and 107-109 as obvious based on Thompson *et al.*, (Eur. J. Biochem., 1990, Vol. 189, pp 73-81) Dobeli *et al.* (US Patent No. 5,310,663) and Ford *et al.* (Protein Expression and Purification, 1991, Vol. 2, pp. 95-107) Applicants respectfully

traverse the rejection.

To establish a prima facie case of obviousness, three basic criteria must be met (MPEP § 2143). First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify a primary reference or to combine reference teachings. Second, there must be a reasonable expectation of success that the suggested combination will work. Third, the prior art reference (or references when combined) must teach or suggest all the claim limitations.

Applicants submit that none of the three criteria have been met.

1. <u>There is no motivation to combine the references.</u>

When applying 35 U.S.C. § 103, the references must be considered as a whole and must suggest the desirability and thus the obviousness of making the combination. *Hodash v. Block Drug Co., Inc.*, 786 F.2d 1136, 1143, n. 5, 229 USPQ 182, 187, n.5 (Fed. Cir. 1986).

Nowhere in the references is there a suggestion of desirability for making a combination to produce the invention as claimed. The Examiner states that "it would have been obvious to one of ordinary skill in the art at the time the invention was filed to be motivated by the recited references to combine the teaching disclosed by Thompson *et al.*, Ford *et al.* or Dobeli *et al.* to make a recombinant Clostridium botulinum type A toxin protein fused with polyhistidine for the convenient large-scale [production and purification] of the recombinant protein...". Applicants note that the Claims specify that the claimed proteins are **soluble**. Nowhere in the Office Action does the Examiner suggest that one

skilled in the art would expect the recombinant *Clostridium botulinum* toxin proteins of the present invention to be soluble. Production of **soluble** recombinant botulinum toxin proteins is an important basis for the present invention.

Prior to the present invention there was no indication that recombinant botulinum toxin proteins could be produced in soluble form. To the contrary, the art at the time of the present invention only taught the production of **insoluble** recombinant botulinum toxin proteins. For example, LaPenotiere *et al.* (Toxicon, Vol 33: 1383-1386 (1995) reported that a (MBP)-botulinum toxin type A C-fragment recombinant fusion protein was produced in **insoluble** form. Therefore, at the time of the present invention there could be no suggestion or motivation in the art to combine references to teach the claimed invention of **soluble**, recombinant *Clostridium botulinum* toxin type A fusion proteins.

2. The cited references do not provide a reasonable expectation of success for the claimed method. For example, the Claims are drawn to **soluble**, recombinant *Clostridium botulinum* toxin proteins. The Ford et al. or Dobeli et al references offer no guidance as to whether **soluble**, recombinant *Clostridium botulinum* toxin proteins would be produced.

In addition, the discovery of the present invention of **soluble**, recombinant *Clostridium botulinum* toxin proteins is in contrast to what had been known in the art at the time of the invention (See, for example, LaPenotiere *et al.* (Toxicon, Vol 33: 1383-1386 (1995)). Therefore, the art, at the time of the invention, actually teaches away from the present invention. This teaching away indicates that based on the prior art, there was no reasonable expectation of success to achieve applicant's invention.

3. The references when combined do not teach each element of the Claims. In particular, the references, alone or in combination, do not teach or even suggest the limitation (element) of "**soluble**" recombinant botulinum toxin protein which is found in the rejected Claims of the present invention.

In summary the cited art does not teach or even suggest **soluble**, recombinant *Clostridium botulinum* toxin proteins. Therefore, the Examiner has not established a prima facie case of obviousness and, accordingly, applicants respectfully submit that the Claims should be passed to allowance.

The Property of "Soluble" Was Not Possessed by the Prior Art

The court has ruled in *In re Papesch* (137 USPQ 43) that the presence of a property not possessed by the prior art is evidence of nonobviousness. The property of "**soluble,**" in reference to a recombinant botulinum toxin protein, was unknown prior to the present invention (See, for example, LaPenotiere *et al.* (Toxicon, Vol 33: 1383-1386 (1995)).

The specification distinguishes between soluble, insoluble and solubilized proteins. It is explained that "soluble," when used in reference to a protein produced by recombinant DNA technology in a host cell, is a protein which exists in solution in the cytoplasm of the host cell. In contrast, an "insoluble" protein is one which exists in denatured form inside cytoplasmic granules (called an inclusion bodies) in the host cell. A "solubilized" protein is a protein that is found in insoluble form (e.g. in inclusion bodies) and is rendered into a soluble form. Solublizing may be done by treating purified inclusion bodies with denaturants such as guanidine hydrochloride, urea or sodium dodecyl sulfate (SDS). These denaturants must be removed from the

solubilized protein preparation to allow the recovered protein to renature (refold). In this process, renaturation is typically inefficient and not all proteins will refold into an active conformation. In addition, many proteins precipitate upon removal of the denaturant. In addition, problems exist with using ionic surfactants to solubilize proteins. For example, during dialysis, SDS can form micelles which do not dialyze out. Therefore, SDS-solubilized inclusion body protein will be solubilized but remain denatured in the presence of SDS. Hence, an insoluble recombinant protein may only sometimes be solubilized and procedures involved in solubilizing are typically inefficient or leave the recombinant protein in denatured form.

It can therefore be seen that the scientific achievement of producing a soluble recombinant protein opposed to an insoluble protein is a significant advantage because of the avoidance of problems encountered with efforts to solubilize insoluble proteins. As the court ruled in (*In re Chupp*, 2 USPQ2d 1437): the presence of an advantageous property is evidence of nonobviousness.

The property of soluble was unknown with respect to recombinant botulinum toxin proteins prior to the present invention. Therefore, applicants respectfully submit that the invention as claimed in not obviousness and the Claims should be passed to allowance.

Solubility was Unexpected

Presence of an unexpected property is evidence of nonobviousness (*In re Chupp*, 2 USPQ2d 1437); (MPEP § 716.02(a)). The Williams declaration (copy attached) presents evidence of an unexpected property associated with the present invention.

The Williams declaration presents significant factual evidence with direct bearing upon the "basic factual inquiries" which must

be made in order to make a proper obviousness rejection, which "basic factual inquiries" include the "the scope and content of the prior art." Graham v John Deere, 383 U.S. 1, 148 USPQ 459 at 467 (U.S. Supreme Court 1966). Additionally, even the limited opinion evidence in the Williams declaration is entitled to deference as the opinion of an expert. *In re Alton,* 37 USPQ2d (Fed Cir. 1996).

Prior to the present invention, when a skilled artisan considered references teaching recombinant botulinum toxin proteins, it is clear that the prior art taught only the production of insoluble recombinant clostridial toxin proteins (see, for example, LaPenotiere, H. et al. (1995) Toxicon, Vol 33: 1383-1386). The insolubility of the LaPenotiere fusion protein was latter confirmed in work by Dr. Williams (Williams' Declaration).

In his declaration, Dr. Williams' explains the unexpected property of solubility of his recombinant botulinum toxin proteins. Dr. Williams was able to obtain, for example, a soluble botulinum type A C-fragment/polyhistidine fusion protein <u>empirically</u> by using, among other things, a relatively weak promoter to drive expression of the fusion protein.

Decisions from the courts which review patent office decisions are instructive as to the deference and weight to be accorded the evidence presented in the Williams declaration: an expert opinion expressed in a declaration can overcome an obviousness rejection: "The expert opinion were introduced on the issue of the level of ordinary skill...the prima facie case of obviousness has been overcome", and the examiner's obviousness rejection was reversed. *In re Oelrich and Divigard,* 579 F.2d 86, 198 USPQ 210 at 215 (CCPA 1978).

Accordingly, applicants submit that the claims relating to soluble recombinant botulinum toxin proteins are not obvious since

the Williams' declaration concludes that the production of **soluble** recombinant botulinum toxin protein was unexpected.

Respectfully submitted,

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MARKED UP VERSION SHOWING CHANGES MADE:

Please change the title to: "SOLUBLE RECOMBINANT BOTULINUM TOXIN PROTEINS."

Please replace Claim 54, 66, 93 and 97 with the following amended Claims:

54. (amended) A soluble, recombinant protein comprising a portion of a *Clostridium botulinum* toxin wherein the portion ranges in size from four amino acid residues to the entire protein minus one amino acid.

66. (amended) A host cell containing a recombinant expression vector, the vector encoding a soluble protein comprising at least a portion of a *Clostridium botulinum* toxin wherein the portion ranges in size from four amino acid residues to the entire toxin minus one amino acid.

93. (amended) A composition comprising a non-toxin protein sequence and a portion of a soluble, recombinant *Clostridium botulinum* toxin wherein the composition is substantially endotoxin-free wherein the portion ranges in size from four amino acid residues to the entire toxin minus one amino acid.

97. (amended) The composition of claim 93 wherein the *Clostridium botulinum* toxin comprises [at least] a portion of SEQ ID NO:28.

CURRICULUM VITAE

James A. Williams, Ph.D.
Vice President, Molecular Biology

BioNebraska Pharmaceuticals, Inc.
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Education:

B. S., Biology with Honors — University of Victoria, Canada, 1981
Ph.D., Genetics — University of Alberta, Canada, 1988

Professional Experience:

BioNebraska, Inc — **11/96 - Present**

Vice President and Director, Molecular Biology &Process Development , BioNebraska, Lincoln. (1998-present)
Vice President and Director, Molecular Biology, BioNebraska, Lincoln. (1997-present)
Director, Molecular Biology, BioNebraska, Lincoln. (1996 - present):

- Project coordinator for Glucagon-like Peptide manufacturing program.
- Director, Molecular Biology, Genetics, Microbiology and Process Development groups.
- Development of recombinant peptide expression technologies.
- Responsible for cGMP manufacture and QC testing of cell banks and peptide pharmaceuticals.

Ophidian Pharmaceuticals, Inc — **10/93 -11/96**

Section Manager, Ophidian Pharmaceutical, Madison. (95-96)
Senior Scientist, Ophidian Pharmaceutical, Madison. (93-94)
Consultant, Ophidian Pharmaceutical, Madison. (93)

- Director, New Technology Discovery group..
- Process Definition of multiple recombinant antigen expression and purification technologies.
- Process Development of recombinant protein fermentation and purification methodologies.
- Developed QC analytical methods for recombinant protein characterization and release testing.

Steritech Pharmaceuticals, INC — **1992**

Consultant, Steritech Pharmaceutical, San Francisco.

- Cloned and expressed recombinant antigens in *E. coli* for diagnostic assays.

Postdoctoral Research — **1989-1993**

University of Wisconsin, Madison with Dr. Sean Carroll. (10/90 - 10/93)
University of British Columbia, with Dr. Tom Griggliatti. (7/89 - 7/90)

- Drosophila research. Multiple Molecular Biology, Biochemistry, Genetic and Immunology projects.

Technical Expertise:

:

Molecular biology, protein over expression and fermentation

- DNA manipulations including cloning and manipulation of M13, plasmid and lambda phage vectors, DNA isolation, Southern hybridization analysis, site directed mutagenesis.
- RNA manipulations including the isolation of RNA, and Northern hybridization analysis.
- Genomic library construction and chromosome walking.
- cDNA library construction and screening.
- cDNA library construction from RNA viruses and cloning of viral genomes.
- DNA sequencing methodologies.
- PCR amplification methodologies.
- Recombinant antigen optimization utilizing protein evolution methodologies.
- Expression vector design, and recombinant antigen expression in *E. coli*.
- 15 liter scale fermentation of E. coli (BioFlo IV, New Brunswick Scientific) and fermentation media and process development.
- Methodologies for processing fermentation cultures (e.g. continuous flow centrifugation, high pressure homogenization, flocculation).

Protein purification and characterization

- Chromatographic purification of proteins (e.g. sizing, affinity, reverse phase, ion exchange chromatography).
- Biochemical analysis of proteins (HPLC, isoelectric focusing, native and SDS-PAGE).
- Protein formulation and stability studies.
- Polyclonal antibody generation.
- Western analysis.
- ELISA or bead agglutination assay development.
- Protein modification (fluorescence, biotin or enzyme labeling of proteins).

Expression vector design

- Construction of proprietary expression vectors and recombinant antigen purification strategies.
- Construction of Production E. coli host utilizing gene disruption/replacement methodologies.

Other

- Immunohistochemistry.
- Immunological screening of expression libraries.
- Protein/protein interaction screens.
- Immunological assay development (e.g. antibody evaluation in complement or phagocytotic killing assays).
- Tissue culture propagation of mammalian cell lines.
- Fluorescence polarization and quenching assays.
- Animal models of infection disease.
- Random peptide library screening.

Regulatory and manufacturing

- Working familiarity with FDA regulations for biological products.
- cGMP peptide pharmaceutical manufacturing experience.
- Development of immunological, microbiological, HPLC and molecular biology Quality Control testing procedures in support of peptide pharmaceutical manufacuring program.
- Drafting of SOP's, specifications and manufacturing batch records.
- Cleanroom setup and establishment of environmental monitoring program.

Publication Record:

Refereed Publications:

Kink, J. A. and **Williams, J. A.** (1998). Antibodies to recombinant *Clostridium difficile* Toxins A and B are an effective treatment and prevent relapse of *C. difficile*- associated disease in a hamster model of infection. *Infection and Immunity* 66: 2018-2025.

Williams, J. A., Paddock, S. W., Vorwerk, K., and Carroll, S. B. (1994). Organization of wing formation and induction of a wing-patterning gene at the dorsal/ventral compartment boundary. *Nature* 368: 299-305.

Carroll, S. B., Gates, J., Keys, D. N., Paddock, S. W., Panganiban, G., Selegue, J. E., and **Williams, J. A.** (1994). Pattern formation and eyespot determination in butterfly wings. *Science*, 265: 109-114.

Williams, J. A., Paddock, S. W. and Carroll, S. B. (1993). Pattern formation in a secondary field: a hierarchy of regulatory genes subdivides the developing *Drosophila* wing disc into discrete subregions. *Development* 117: 571-584.

Bazin, C., **Williams, J.**, Bell, J. and Silber, J. A. (1993). A deleted *hobo* element is involved in the unstable thermosensitive vg^{al} mutation at the vestigial locus in *Drosophila melanogaster*. *Genetical Res.* 61: 171-177.

Clegg, N. J., Whitehead, I. P., **Williams, J. A.**, Spiegelman, G. B. and Grigliatti, T. A. (1993). A developmental and molecular analysis of *Cdc2* mutations in *Drosophila melanogaster*. *Genome* 36: 676-685.

Heslip, T. R., **Williams, J. A.**, Bell, J. B. and Hodgetts, R. B. (1992). A *P* element chimera containing captured genomic sequences was recovered at the *vestigial* locus in *Drosophila* following targeted transposition. *Genetics* 131: 917-927.

Williams, J. A., Bell, J. B. and Carroll, S. B. (1991). Control of *Drosophila* wing and haltere development by the nuclear vestigial gene product. *Genes Devel.* 5: 2481-2495.

Williams, J. A., Scott, I. M., Atkin, A. L., Brook, W. J., Russell, M. A. and Bell, J. B. (1990). Genetic and molecular analysis of vg^{U} and vg^{W}: two dominant *vg* alleles associated with gene fusions in *Drosophila*. *Genetics* 125: 833-844.

Williams, J. A., Atkin, A. L. and Bell, J. B. (1990). The functional organization of the *vestigial* locus in *Drosophila melanogaster*. *Mol. Gen. Genet.* 221: 8-16.

Williams, J. A. (1988). A molecular analysis of the *vestigial* locus in *Drosophila melanogaster*. Ph.D thesis, University of Alberta, Edmonton, Alberta.

Williams, J. A., Pappu, S. S. and Bell, J. B. (1988). Suppressible *P*-element alleles of the *vestigial* locus in *Drosophila melanogaster*. *Mol. Gen. Genet.* 212: 370-374.

Williams, J. A., Pappu, S. S. and Bell, J. B. (1988). Molecular analysis of hybrid dysgenesis-induced derivatives of a *P*-element allele at the *vg* locus. *Mol. Cell. Biol.* 8: 1489-1497.

Williams, J. A. and Bell, J. B. (1988). Molecular organization of the *vestigial* region in *Drosophila melanogaster*. *EMBO* 7: 1355-1363.

Molnar, C. M., Reece, T., **Williams, J. A.** and Bell, J. B. (1988). Transformation of *Drosophila melanogaster* with a suppressor tRNA gene (Sup3e $tRNA^{Ser}$) from *Schizosaccharomyces pombe*. *Genome* 30: 211-217.

Book Chapters and Review Articles:

Williams, J. A., Langeland, J. A., Thalley, B., Skeath, J. B. and Carroll, S. B. (1995). Production and

purification of polyclonal antibodies against proteins expressed in *E. coli*. DNA Cloning: Expression Systems, IRL Press.

Williams, J. A. and Carroll, S. B. (1993). The origin, patterning and evolution of insect appendages. *Bioessays* 15: 567-577.

Patents:

Williams, J. A., Kink, J. A., Clemens, C. and Carroll, S. B. *Clostridium difficile* toxin disease therapy. U.S. Patent 5,762,934 issued 6/98.

Williams, J. A., Kink, J. A., Clemens, C. and Carroll, S. B. Avain antitoxins to *Clostridium difficile* toxin A. U.S. Patent 5,601,823 issued 2/97.

An additional 8 patent applications are pending at the US Patent office.